Preventing the Introduction of Plant Pathogens: The Role and Application of the “Systems Approach”

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I. Purpose

The purpose of this study is to satisfy, in part, the requirements of Title IV, Section 412(e) of the Agricultural Risk Protection Act of 2000. The Act requires the conduct of a study and submission of a report, not later than two years after its enactment, on the “role for and application of Systems Approaches designed to guard against the introduction of plant pathogens into the United States associated with proposals to import plants or plant products into the United States.”

II. Agency Responsibility

The USDA, APHIS, PPQ (PPQ) is responsible for safeguarding America’s agriculture and natural resources. The Plant Protection Act (PPA) authorizes PPQ’s safeguarding activities. Various federal foreign quarantines have been promulgated in Title 7, Code of Federal Regulations to address specific plant pests and pest risks.

The PPA and federal foreign quarantine requirements are enforced at air, land and sea ports of arrival in the United States and at points of origin where pest host commodities are produced, harvested, packaged, and assembled for shipment to the United States. The USDA, APHIS, International Services (IS) assists the PPQ at foreign country export points where IS offices are established.

III. Background

Global trade in plant commodities, increased travel for business and pleasure, foreign country economic development initiatives, and other human endeavors have given rise to a number of pivotal agreements aimed at eliminating artificial barriers to trade and protecting natural resources. The General Agreement on Trade and Tariffs (GATT), North American Free Trade Agreement (NAFTA), World Trade Organization’s Sanitary and Phytosanitary Agreement (SPS), Biodiversity Convention and Biodiversity Protocol are significant examples.

Under the SPS, countries, consistent with specific disciplines, may exercise their sovereign rights to protect their agriculture, natural resources and economy against the harm that quarantine pests could cause. Importing countries may establish what they determine to be their acceptable level of risk. The appropriateness of their acceptable level of risk is judged in accordance with the SPS disciplines and other relevant factors.

Pest risk analysis is the method that is to be used to determine the level of pest risk associated with the importation of a specific commodity, together with the risk associated with the pathway for that commodity. Upon determining the level of pest risk and the acceptable level of risk, the importing country evaluates the available methods for reducing the risk to an acceptable level.

Imports from areas that are free of quarantine pests represent the ideal situation for ensuring that agricultural, natural resources and economic interests are protected. However, when a quarantine pest is known to be present, imports are either prohibited or restricted to areas where adequate safeguards have been applied.

IV. Historical Plant Pest Risk Mitigation Practice

In the past, when quarantine pest risks were identified, it was customary for importing countries to rely on very highly effective chemical or other treatments to achieve what was called Probit 9 security. No other treatments or risk reduction methods were deemed necessary to achieve an acceptable level of protection.

For example, citrus and mangos imported from Mexico, where the Mexican fruit fly (*Anastrepha ludens*) occurs, were treated in a fumigation chamber using a prescribed dosage of ethylene dibromide applied according to a prescribed time, temperature and air circulation protocol. Registration for the use of ethylene dibromide was cancelled in the 1980s.

In response to cancellation of the registration for ethylene dibromide, the Mexican government collaborated with citrus growers and the USDA to develop and approve pest free areas in the State of Sonora. Hot water treatment has been substituted for ethylene dibromide fumigation for mangos. Methyl bromide fumigation is an alternative treatment for Mexican fruit fly used by grapefruit growers and shippers in the southern Rio Grande Valley of Texas in the United States.

V. The Systems Approach To Plant Pest Risk Mitigation

The loss or lack of single treatment methods for reducing pest risk to an acceptable level has led to combining treatments, each of which reduces pest risk by some level, to achieve an overall acceptable level of risk for pests that infest a particular commodity and for pests that might move incidentally with that commodity. This approach to pest risk mitigation has been called the "Systems Approach." The term was first used in 1994 to describe an insect management system developed to reduce for importation of avocados from Mexico (Miller et. al, 1995).

This study uses the definition of Systems Approach found in the Plant Protection Act [7 USC 7712 (e) Section 412 (e)] and described as:

> "[A] defined set of phytosanitary procedures, at least two of which have an independent effect in mitigating pest risk associated with the movement of commodities."

A May 2001 United Nations Food and Agriculture Organization, International Plant Protection Convention draft "International Standards for Phytosanitary Measures" (ISPM) dealing with the Systems Approach is entitled, "Integrated Measures for Pest Risk Management (Systems Approaches)." This draft document defines "System Approach" as:

> "[T]he integration of different pest risk management measures, at least two of which act independently, and which cumulatively achieve the desired level of phytosanitary protection."

While the definitions vary slightly and any number of measures may be included, the key point is that at least two of the selected measures must have an independent *and* additive effect on reducing the risk of pest introduction. Any other measures may be inter-dependent. If the success of one measure is influenced by the success or failure of another measure, neither is independent. For example, sampling to determine if a pathogen is present and applying a pesticide if the pathogen is detected are not independent actions. If the sampling failed to detect an existing population, resulting in a decision to not apply the pesticide, both mitigation measures failed.

On the other hand, two independent measures would be 1) sampling to detect pathogen populations combined with 2) mandatory application of pesticide based on environmental conditions favoring infection.

In addition, there might be pest situations where multiple independent control measures would be incorporated to: 1) ensure effectiveness, 2) address some level of uncertainty associated with the efficacy of inter-dependent measures or a particular measure in the system, or 3) take into consideration conditions or factors not entirely predictable or that could vary in such a way as to reduce effectiveness of a measure or the system as a whole. (IPPC draft July 27, 2000 Brisbane).

Another principle is equivalency. Equivalency recognizes that certain mitigation measures, while not identical, can have the same effect. This implies that the desired effect can be clearly defined and measured (quantitatively or qualitatively) and that there are various options that can be used to achieve the desired effect.

The Systems Approach can be likened to an extended IPM program. Management strategies can be applied at any time from pre-plant selection or treatment of the growing area and selection of pest-free planting material through growing season management, post-harvest handling and storage, and shipping to wholesale and retail outlets for distribution to the consumer.

Measures such as chemical treatments applied to growing crops and post-harvest fumigation, cold or heat treatment, or controlled atmosphere storage of fruits can be used to kill certain quarantine pests. However, determining their efficacy in killing or rendering plant pathogens non-viable is critical, complex and uncertain. Other measures, such as host resistance and pest-free growing areas, can be used to exclude a pathogen from an export commodity. Still other measures, such as restricted shipping times and destinations (i.e. import of tropical fruits only to the New England states and only in winter), reduce the potential for the pathogen to become established in the U.S., even if it should be present in or on the commodities at the time they enter the U.S.

VI. Application of the Systems Approach to Plant Pathogens

While the term Systems Approach has only been used since 1994, the concept and use of multiple management practices to guard against the introduction of plant pathogens and other food-borne pests has been in use since the 1960's (IPPC Secretariat Discussion Paper, January 1999; Jang and Moffitt, 1994). The protocol required to export Unshu oranges from Japan to the U.S. is a well-known example that has been in use for more than 20 years.

Several others, including various fruit and ornamental trees from Europe, chrysanthemums from various countries, and carnations from the United Kingdom, were initiated in 1980. In 2000, a Systems Approach was approved for the importation of citrus from Argentina.

To date, no APHIS PPQ-approved Systems Approaches to safeguard against plant pathogens have failed or been discontinued (Burnett report, 2001). Furthermore, no plant pathogens have been introduced when the importation of plant material or plant products was managed according to an APHIS PPQ-approved Systems Approach.

VII. Theoretical Foundation

The goal of any nation's phytosanitary import regulations is to prevent entry *and* establishment of exotic or non-indigenous organisms, including plant pathogens that pose a risk to plant life or health. Either entry or establishment must be prevented. A Systems Approach employs independent mitigation measures targeting either or both entry *and* establishment.

Relative to entry, importation and distribution of a commodity within areas where conditions are suitable for establishment of a targeted pathogen would be reasonably safe provided that there was a high degree of confidence that a targeted pathogen would be detected and eliminated at origin.

In the case of establishment, if distribution of an imported commodity were to be limited to an area where the commodity is not grown and there are no other hosts for the targeted quarantine pathogen, it would be less critical to detect and eliminate the pathogen before entry of the commodity. This presumes that the commodity will not trans-shipped to areas where host material is present.

The focus of the Systems Approach, as with other risk mitigation measures, is to reduce the probability of entry or establishment of a targeted pest to an acceptable level. There is no expectation that the possibility of introduction can be entirely eliminated. Simply stated, there is no such thing as "zero risk." So long as human enterprise and trade continue, no individual control measure can be guaranteed to be 100% effective. Risk mitigation efforts, for instance, rarely address the introduction of a targeted pest that might result from smuggling.

Any importing nation must determine the acceptable probability level of excluding a specific pathogen. Mitigation measures can then be evaluated to determine if they provide the required probability level. Some phytosanitary measures, such as methyl bromide fumigation are one-step mitigation measures. Scientific data and years of experience have shown that this treatment can provide an acceptable level of protection for certain commodity/pest combinations. Unfortunately, some commodities are damaged by chemical fumigants, therefore other mitigation strategies are needed to allow the exchange of commodities.

The Systems Approach requires two or more *independent* control or mitigation measures. If only one measure is required, and it fails, the pathogen gains entry. If multiple independent mitigation measures are used, they form a pyramid, each measure building on the prior measures and increasing the probability of preventing the entrance and establishment of an unwanted pathogen. This is an approach that is used in many aspects of life. To prevent loss of our household possessions, we lock the doors and windows. Just in case that isn't enough, we install a burglar alarm system. For even greater safety, we keep a big, loud dog in the house. Each measure increases the probability that the contents of our home will be safe.

The Systems Approach to prevent the introduction and establishment of plant pathogens is very similar. Before walking through a simple example, a review of probability theory is needed. Events can be mutually exclusive, there is no overlap (Fig. 1). An example would be a jar containing 2 red marbles, 3 blue marbles, and 5 yellow marbles for a total of 10 marbles in the jar. Each individual marble is either red, or blue, or yellow. There is no overlap. The probability of picking a red marble out of the jar is 2/10 or P(red) = 0.2. The probability of picking a yellow marble is 5/10 or P(yellow) = 0.5. The probability of picking a red

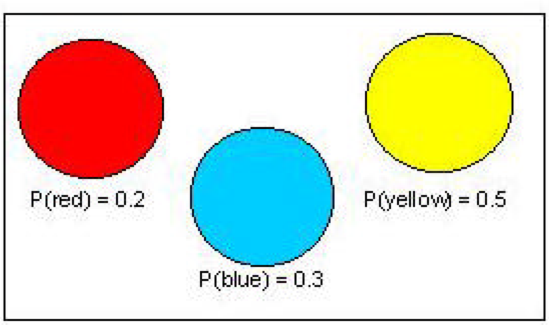


Figure 1. Mutually Exclusive Events – no overlap.

marble OR a yellow marble is P(red) + P(yellow) = 0.2+0.5 = 0.7.

An example of a mutually exclusive event in plant pathogen control would be individual fruits, plants or units in a shipment or consignment of a plant commodity that are either infected or not infected. The unit cannot be both infected and uninfected. There is no overlap. The pathogen is either detected then eliminated by a mitigation measure OR it is not. Let a jar of 100 marbles represent a consignment or shipment of a particular commodity. Twenty red marbles represent the individual fruits, plants or other discrete units (cartons, bags, etc.) that are infected or infested, as the case may be. The pest prevention goal is to reduce the number of individual infected fruits, plants or other units to an acceptable level. Suppose that the acceptable level is determined to be only one infected fruit, plant or unit. In the Systems Approach, the question is what combination of independent measures can be applied to ensure that no more that one fruit, plant or unit in each consignment will be infected or infested.

Events can overlap (Fig 2.). If a jar has 10 marbles 60% of which are blue and 40% are red and 50% of each color are clear and 50% are opaque, the probability of drawing a blue marble is P(blue) = 0.6. The probability of drawing a clear marble is P(clear) = 0.5. The probability of drawing a marble that is blue AND is clear is P(blue AND clear) = P(blue) x P(clear) = 0.6 x 0.5 = 0.3. Another way of thinking of this is that of the 60% that are blue, 50% (or half) of the blue are also clear. Half of 6 is 3, so there are 3 marbles, out of 10, that are both blue and clear. If some of the marbles are small and some are large, the probability of a small, blue, clear marble = P(small) x P(blue) x P(clear). In quarantine arenas, a single crop unit (fruit, plant, carton or bag) within a shipment could be infected with a pathogen AND detection of the pathogen could fail. If the probability of being infected is P(infected) = 0.05 and the probability of the mitigation measure failing is P(mitigation failed)= 0.1 then the probability of the pathogen being present but not eliminated by the mitigation measure is P(present AND mitigation failed) = P(present) x P(mitigation failed) = 0.05 x 0.1 = 0.005.

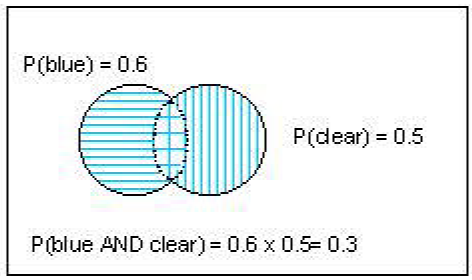


Figure 2. Overlapping events. One circle represents blue marbles. The other represents clear marbles. The overlap are marbles that are both blue AND clear.

If we are interested in all instances in an overlapping events scenario in which EITHER OR BOTH events occur, the diagram resembles Figure 3. We want to include all of both circles. If we add the 2 probabilities together, as we did in Figure 1, we would count the overlap area twice – once as being part of the blue circle and again as being part of the clear circle. The sum would give us a clearly impossible probability of 1.1. In Figure 2, we calculated the probability of the overlap as P(blue) x P(clear). If we now subtract the value of the overlap from the sum of the two circles, we will count the overlap area only once. P(blue OR clear) = P(blue) + P(clear) – (P(blue) x P(clear)) = 0.6 + 0.5 – (0.6 x 0.5) = 1.1 - 0.3 = 0.8. A piece of fruit can become infected from one or more exposures to a plant pathogen. If the probability of a pathogen infecting a commodity in a area of low pathogen occurrence is P(pathogen in low-occurrence zone) = 0.05 and the probability of pathogen-infected planting material being certified as pathogen-free is 0.01, then the probability of commodity becoming infected as a result of a pathogen entering the area and/or coming in on certified clean planting material is 0.05 + 0.01 - (0.05 x 0.01) = 0.06 - 0.0005 = 0.0595.

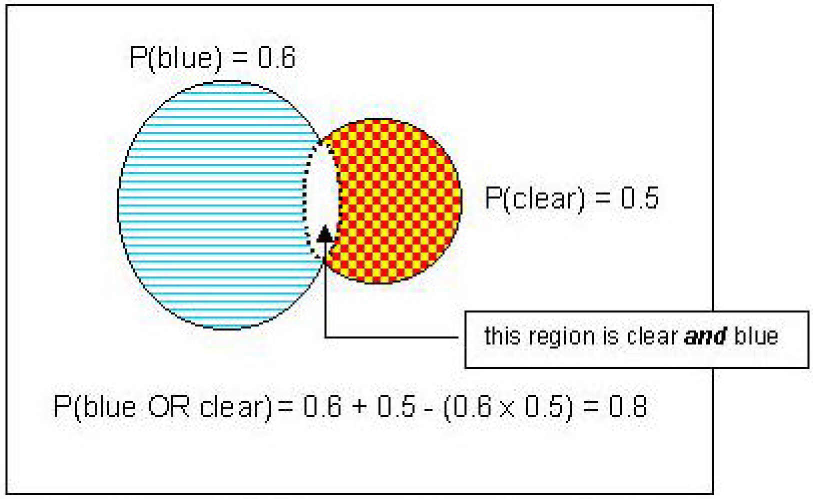


Figure 3. The probability of being either blue or clear is equal to the combined probability of both minus the overlap.

Table 1 compares the probability of successful detection/elimination of a plant pathogen using a single mitigation measure with the success that can be achieved using multiple mitigation measures as in the Systems Approach. This case illustrates the strengths of the Systems Approach:

> The use of certified propagation materials (mitigation measure #1) has a probability of successfully eliminating the hypothetical plant pathogen of 0.90, meaning the probability of the pathogen being introduced by a failure of the certified propagation material component is 0.10. If this were the only mitigation measure, the probability of failure is 0.10.
>
> Used alone, Mitigation measure #2 (in-field chemical application) has a theoretical probability of success of 0.80. Mitigation measures #1 and #2 are independent of each other. Used together, the probability of successful elimination due to measure #2 is applied to the 0.10 failure following the first mitigation measure. The resulting probability of success would be 0.08 (0.80 x 0.10 = 0.08). Adding this 0.08 to the

0.90 eliminated by measure #1, results in a probability of successfully eliminating the pathogen of 0.90 + 0.08 = 0.98 if both measure #1 and measure #2 are used.

Using two measures clearly provides a greater degree of safety than using either measure alone. Each additional independent mitigation measure increases the probability of successfully detecting and eliminating the target pathogens.

Table 1 shows the increasing probability of successful detection or pathogen elimination as each additional measure is applied. This multi-step approach clearly provides an increasing level of security as compared to single mitigation approaches. In order for a pathogen to enter and become established, the commodity being imported would have to be infected AND all mitigation measures would have to fail. The probability of this happening is reduced with each additional independent mitigation measure incorporated into the management system.

In the hypothetical example given in Table 1, after the application of the six mitigation measures, the probability of the hypothetical pathogen entering and becoming established is 0.0000003. Is this an absolute guarantee that the system won't fail? No! Only that the probability of failure is extremely low. No absolute guarantee possible is possible.

Table 1. Comparison of the probability of successful detection/elimination of plant pathogens using a single mitigation measure versus multiple independent mitigation measures (Systems Approach).

Hypothetical Mitigation Measure	Single Component		Cumulative Systems Approach	
	Probability of Successful Elimination or Detection	Probability of Failed Elimination or Detection	Cumulative Successful Elimination or Detection	Cumulative Failed Elimination or Detection
1- Certified Propagation Materials	0.90	0.10	0.90	0.10
2- In-field Chemical Application	0.80	0.20	0.90 + (0.80 x 0.10) = 0.98	0.20 x 0.10 = 0.02
3- Pre-harvest Sample and Incubation to Detect Latent Infections	0.85	0.15	0.98 + (0.85 x 0.02) = 0.997	0.15 x 0.02 = 0.003
4- Post-harvest surface sterilization in the pack house	0.90	0.10	0.997 + (0.90 x 0.003) = 0.9997	0.10 x .003 0.0003
5- Port of Entry Inspection	0.95	0.05	0.9997 + (0.95 x 0.0003) = 0.999985	0.05 x .0003 = 0.000015
6- Restricted Distribution to Areas Unsuited for Pathogen Establishment	0.98	0.02	0.999985 + (0.98 x 0.000015) = 0.9999997	0.02 x 0.000015 = 0.0000003

This example is for a series of sequential mitigation measures. The additive effects are sequence-specific with each succeeding mitigation effect being a probability conditional upon the preceding mitigation effect(s). Other examples of Systems Approaches have simultaneous action of independent measures.

VIII. Designing A Systems Approach

Data and Knowledge Base Requirements

Systems Approaches will generally be more difficult to develop and implement than a probit-9 post-harvest treatment. (This will depend on how much biological, risk mitigation, and other information is already known.) Each Systems Approach for a crop/pest complex is likely to be a unique assemblage of tactics because the dynamics of a pest complex are shaped by the biology of the crop (host) and related biological complex, its soil climate regime (agro-eco-region) and local agronomic practices. Yet, the Systems Approach may have less negative impact on commodity quality or be less trade restrictive than other risk mitigation measures, especially where the alternative is a prohibition of importation.

A joint workshop of USDA ARS and USDA APHIS identified the conditions that allow for a successful Systems Approach to be developed (Liquido et. al, 1997):

- Pests associated with the commodity are known;
- Basic biology of the pest(s) is known, including pest/host relationship, dispersal, alternate hosts, habitat selection, and population dynamics;
- Knowledge of the pathogen and disease life cycle;
- Systems exist for field surveillance and/or detection of pests in shipment;
- Knowledge of harvesting, packing, and marketing practices exists;
- Pest(s) are generally absent or rare in commercial commodity because of :
    1) normal field management;
    2) poor host;
    3) resistant cultivar;
    4) phenological asynchrony between pest and commodity; or
    5) ecological limitation-based rarity of pest in growing area no alternative method is available for obtaining phytosanitary security; or a Systems Approach is more desirable because it does not damage the commodity and/or is more cost-effective;
- Sufficient volume of the commodity is shipped to justify and offset the program costs;
- Some degree of redundancy and independence between program components can be designed to allow for variability in pest populations or partial failure of other components; and
- Phytosanitary security is apparent either by qualitative or quantitative assessment.

The development of a Systems Approach must be undertaken in a methodical manner making the best use of the knowledge of the pathogen and host biology, pathogen ecological requirements, the marketing and distribution system, and the level of risk acceptable to the importing country. It is important to realize that a control measure must be both effective and practical. Systems approaches have been developed for both propagative plant material and for plant products (fresh fruit). A two-year post-entry quarantine might be both effective and reasonable to guard against viruses in fruit trees, but would clearly not be acceptable for a perishable commodity.

The International Plant Protection Convention (IPPC) developed a set of steps to be taken to develop and implement a Systems Approach (IPPC, 2000):

- Identify the pest risk, pathway risk;
- Describe the pathway;
- Identify where management measures occur or can be applied;
- Distinguish essential measures and other factors or conditions;
- Identify independent and dependent measures and redundancy;
- Assess the individual and integrated efficacy of essential measures;
- Assess feasibility and trade restrictiveness;
- Consult and negotiate with importing country;
- Implement with documentation and reporting; and
- Review and modify as necessary.

Components of a Systems Approach

The components available for inclusion in a Systems Approach run the entire gamut of pathogen management, but can generally be divided into four categories:

1) exclusion of the pathogen,
2) detection of the pathogen, (detection alone is not a management or risk mitigation measure) and
3) elimination of detected pathogen populations, or
4) reduced risk of establishment in the importing region.

Common risk mitigation measures include (IPPC, 2000):

Pre-harvest

- Field certification/management (treatments, biocontrol, etc);
- Protected conditions (glasshouse, fruit bagging, etc);
- Resistant or less susceptible cultivars;

- Harvesting plants at certain age or time of year;
- Vector mating disruption (particularly effective with insects);
- Cultural controls;
- Vector- and pathogen-free areas, places or sites of production;
- Low prevalence (continuous or at specific times); and
- Testing and subsequent elimination of infected components

<u>Harvest</u>

- Culling, inspection or selection;
- Stage of ripeness/maturity;
- Timing of harvest;
- Sanitation (e.g. – removal of reservoir hosts, "trash"); and
- Harvest technique, handling.

<u>Post-harvest treatment and handling</u>

- Treatment to kill, sterilize or remove vectors or pathogens (fumigation, irradiation, cold, controlled atmospheres, washing, brushing, waxing, dipping, heat, etc.);
- Inspection and grading;
- Sanitation, including removal of parts of the host;
- Certification of packing facilities; and
- Testing with subsequent elimination of infected component.

<u>Shipping and distribution</u>

- In-transit or on-arrival treatment or processing;
- Restrictions on end use, distribution and periods; and ports of entry restrictions;
- Post-entry quarantine;
- Inspection and/or testing with subsequent elimination/denial of entry;
- Speed and type of transport ; and
- Sanitation (freedom from contamination of carriers).

Minimum criteria for a measure to be considered a required component in a Systems Approach are that the measure: 1) is clearly defined; 2) is found or known to have a specific level of efficacy; 3) is officially required (mandatory); and 4) can be overseen and controlled by the responsible National Plant Protection Organization (NPPO)(IPPC draft July 27, 2000).

The IPPC further describes three structural approaches that can be used as the framework for a Systems Approach:

- Mitigation Systems – a combination of official phytosanitary procedures.
- Quality Systems – mix of phytosanitary procedures and other procedures. Typically, these include a range of processes designed to ensure the quality of commodities, but also contribute to phytosanitary security.
- Control Point Systems – equivalent of the Hazard Analysis Critical Control Point (HACCP) used in food safety. This involves rigidly defined independent events or processes that are measured, monitored, and controlled.

IX. Assessing Systems Approach Performance (Verification)

In the end, the success of the Systems Approach will be measured by its ability to achieve a defined level of phytosanitary security. Phytosanitary security results from the application of single phytosanitary measures in a specific situation and may be evaluated quantitatively or qualitatively according to the defined endpoint.

Single tactic disinfection treatments as a rule overkill significantly so as to ensure that the probability of a target pest surviving is very low. For example, methyl bromide fumigation may provide kill rates of 99.9968% of treated individuals. While Probit 9 provides a standard for the evaluation of a single treatment as a risk mitigation method, new measures are needed for assessing the performance of particular Systems Approaches. Basically, the importing country makes the final determination of what constitutes adequate quarantine security. In practice, the exporting country develops the Systems Approach for a particular quarantine pathogen(s) and then proposes it to the importing country. The result is a negotiated level of quarantine security, which could be lower than, higher than or equal to Probit 9.

Such negotiations can only take place when the qualitative or quantitative degree to which the particular measures making up the Systems Approach are known or can be calculated. The same is true in terms of performance assessment or verification (compliance determination) by the importing country.

Interventions in a well-designed Systems Approach should be additive or synergistic. For example, systems are comprised of control tactics with an associated efficacy and by events that can lead to infestation. Those events may include orchard floor conditions, fruit falling to the ground, or condition of the packing area (i.e. - pest-free for some period of time). Probabilities of infestation can be calculated for each of these events or possible points of infection by a pathogen.

As a specific case example, a chemical treatment for the avocado seed weevil will have an associated efficacy. The probability of a pest complex infesting an avocado is increased by fruit falling to the ground. In the case of avocado, the mortality resulting from a combination of treatments was estimated by multiplying the effects of individual treatments (Finney, 1971; Couey and Chew, 1986; Robertson and Preisler, 1992) and reviewed in Mangan and Sharp (1994). The expected mortality (M) for a series of treatments (t_1, t_2, t_3: t_1 = survival rate) was calculated as follows (where 1-t is the mortality rate):

$$M = 1 - (1 - t_1)(1 - t_2)(1 - t_3)$$

Any number of treatments could be included in this approach. This expected mortality would then be compared to experimentally derived measures of mortality to evaluate the additive effects of combined treatments.

Inasmuch as a Systems Approach involves both treatments and events in the management of risk, a probabilistic method may be applied. Treatments can be evaluated and assigned a probability to reduce potential pest risk. Similarly, the probability to reduce potential pest risk can be estimated or measured for the effectiveness of field sanitation, host resistance, post-harvest safeguards etc. The reality is that there is variation associated with any of these measures, and for this reason, a range of values is often reported and used in such analyses.

Once the necessary risk reduction matrix is constructed, the probability that phytosanitary security will be achieved can be estimated (under a range of assumptions, i.e. - best case, worst case) in a fashion similar to that described above. The probability that a Systems Approach fails to provide an agreed upon level of phytosanitary security (P_{SA}) can be estimated using the following equation (where P_{SA} is the probability that the target pathogen will be detected and eliminated and ($1\text{-}P_N$) is the probability of an independent mitigation measure failing to detect and eliminate the pathogen):

$$P_{SA} = 1 - (1\text{-}P_1)(1\text{-}P_2)\ldots(1\text{-}P_N)$$

In this approach, the probabilities are not additive. Rather a high probability of any one measure failing will compromise phytosanitary security. To illustrate the elements of a Systems Approach and the range of probabilities estimated for each element see Table 2, where elements for a Mexican avocados Systems Approach were estimated. The table clearly shows the many intervention points in managing phytosanitary security. In reviewing this table, keep in mind that the knowledge base needed to derive such a table is considerable.

Table 2 - Systems Approach: Mexican Avocado*

Risk Mitigation Measures	Reduction of Potential Pest Risk					
	Fruit Flies: *Anastrepha spp.*	**Small avocado seed weevils:** *Conotrachelus spp.*	**Avocado stem weevil:** *Copturus aguacatae*	**Large avocado seed weevil:** *Heilipus lauri*	**Avocado seed moth:** *Stenoma catenifer*	**Hitchikers and other pests**
Field Surveys	40% to 60%	95% to 99%	80% to 95%	95% to 99%	95% to 99$	40% to 75%
Field Sanitation	75% to 95%	15% to 35%	70% to 90%	15% to 35%	15% to 35%	20% to 40%
Host Resistance	95% to 99.9%	0	0	0	0	0
Post-harvest Safeguards	60% to 80%	0	0	0	0	40% to 60%
Winter Shipping Only	60% to 90%	0	0	0	0	50% to 75%
Packnghouse Inspection and Fruit Cutting	25% to 40%	50% to 75%	40% to 60	50% to 75%	50% to 75%	30% to 50%
Port of-Arrival Inspection	50% to 70%	50% to 70	5%0% to 70	50% to 75%	50% to 75%	60% to 80
Limited U.S. Distribution	95% to 99%	95% to 99%	90% to 99%	95% to 99%	95% to 99%	75% to 95%

* From "Risk Management Analysis: A Systems Approach for Mexican Avocado" (USDA-APHIS)

X. Case Studies

Historically, plant pathogen introductions were managed by not importing from regions of the world where the pathogen was known to occur. With a loosening of trade restrictions, more and more often the pest-free status is seen as too restrictive. Systems approaches have been designed in a number of crops (Table 3) as an effective means of facilitating trade while at the same time minimizing the risk of introduction and establishment of unwanted plant pathogens.

The following six case studies were chosen to serve as examples of system approaches of varying complexity. Some have been in use for over twenty years and others have only recently been initiated. They vary from targeting individual pathogens to multiple pathogens within a single geography.

Table 3. Systems approaches currently in place to guard against the introduction and establishment of plant pathogens in the U.S.

- Unshu oranges from Japan and Korea– initiated 1967.
- Plant growing in media – initiated 1980.
- Various fruit and ornamental trees from Europe - initiated 1980.
- *Rubus* from Europe - initiated 1980.
- Chrysanthemum plants from various countries - initiated 1980.
- Carnations from UK – initiated 1980.
- Grapevines from Canada – initiated 1992.
- Grafted lilac from Netherlands – initiated 1992.
- *Gladiolus* from Luxembourg or Spain – initiated 1992.
- Irish potato true seed – initiated 1995.
- Ya pears from China – initiated 1995, and
- Citrus from Argentina – initiated 2000.

True Potato Seed From Chile's 'X' Region: Interdiction Of Viral Pathogens

Potato black ringspot virus (PBRSV) (also known as *Tobacco ringspot virus* Andean calico strain) is one of at least five viruses identified as infecting *Solanum tuberosum*, wild *Solanum* spp. and weed hosts found in the Andean highlands of South America. While authorities disagree whether this virus transmits via true potato seed, the perceived threat of introducing this or similar viruses to the United States via breeding stock warranted a long-standing embargo against the import of this commodity to the United States from any nation except Canada. In fact, the prohibition on import of potato seed was only relaxed in 1995 and then, only for breeding stock exported from a narrowly defined region in Chile.

This case study describes the implementation of a Systems Approach to reduce the risk of introducing PBRSV. The same approach has also been employed to prevent introduction of *Andean potato latent tymovirus* (APLV), *Arracacha B 'nepovirus'* oca strain (AVB-O), *Potato yellowing alfamovirus* (PYV), and *Potato T trichovirus* (PVT). No single component of the Systems Approach described here is uniquely designed to prevent importation of PBRSV. Rather, when combined, these components aim to prevent transmission of any one of the five viruses listed above. For this study, then, PBRSV exemplifies the other four target viruses listed above. Yet, each of these viruses is a unique pathogen exhibiting a variety of symptoms, disease cycles and economic significance.

Distribution

The only confirmed reports of PBRSV are in Peru and Chile (along the mountainous border between the nations), but authorities suspect the virus may be present, though unidentified, in other Andean countries. The distribution of the other five viruses is similar. There are confirmed cases of each virus in Peru, and confirmed cases of APLV reported in Bolivia, Columbia, Uruguay, Paraguay and Argentina. Figure 4 shows the known distribution of each of the five viruses listed above.

Symptoms

Under Andean highland conditions, several cultivars of *Solanum tuberosum* develop calico-like symptoms when infected by PBRSV. Bright yellow areas on the margins and upper leaves gradually increase in size to form large patches. Most of the plant foliage may eventually turn yellow without stunting or leaf deformations. Plants that become infected during the current growing season show local and systemic necrotic spots and ringspots. Sometimes systemic necrosis also is observed.

Symptoms of PBRSV are persistent and under certain conditions and in some cultivars, the virus causes serious damage to the crop.

Disease Cycle

PBRSV is thought to be transmitted by a vector, though none has been conclusively identified. As noted above, there is ample reason to be concerned that it is transmitted through true potato seed. The virus spreads locally by contact between plants and possibly through insect or nematode vectors.

International Dissemination

This virus is most likely to spread across geo-political boundaries as a result of trade in seed potatoes used by professional breeders. Because of the probability that any material of wild tuber-forming *Solanum* spp. originates ultimately from South America, the import of either tubers or true potato seed into the United States is strictly limited almost without regard to the country of export.

Control

As with all potato viruses, control of PBRSV depends on the production of high-quality seed potatoes from virus-free nuclear stock.

Systems Approach

Preventing the transport of the PBRSV and the other viruses described above hinges on establishing a method for producing virus-free potato seed. Since it is unclear what vectors might transfer PBRSV from neighboring hosts to plants being cultivated for export of germplasm, this pathogen serves an excellent example of how a Systems Approach can be implemented.

The mitigation steps followed in Chile's 'X' region are as follows (Fig. 5):

Parent-Plant Propagation System

- Parental lines destined for Chilean production sites are developed in California, through a breeding program. Tubers from parent plants are disinfected and then stored until dormancy break. Tubers are then moved to a dark area until sprouting and disinfected again.
- This material is transferred to a quarantine area where meristematic tissue is taken from the sprout and cultured under aseptic conditions. The source of each tissue culture is accurately recorded, and the tissue is subcultured twice to provide sufficient material for virus testing. After tissue culturing, three individual plantlets are subjected to serological testing for 16 different viruses, including the five target viruses: PBRSV, APLV, AVB-O, PYV and PVT.
- Rapid multiplication of the virus-free plantlets occurs through micropropagation, and 1% of these plantlets are again tested for virus before shipment to Chile.

Micropropagation (at Chilean location)

- Micropropagation taking place in Chile is performed using aseptic methodology.
- Again a 1% sample of the micropropagated plantlets is tested for all 16 viruses.

Greenhouses

- Plantlets are transplanted in greenhouses located in isolated areas known to be free from traditional potato diseases.

- Greenhouse substrate is sterilized with steam.
- Aseptic procedures are followed in greenhouses.
- Greenhouses are equipped with window and door screens designed to prevent the entrance of aphids and other potential vectors.

<u>Virus Testing</u>

- Minitubers obtained from the greenhouse are virus tested.

<u>Fields</u>

- The fields where the male and female plants grow are located in a unique region of Chile (Region 'X'), which is naturally isolated from neighboring potato growing regions. Chile is bounded by the Pacific Ocean to the west, a desert to the north, ice fields to the south, and the Andes Mountains to the east. Region 'X' is further isolated from potato viruses by the Chilean Ministry of Agriculture's strict controls over agricultural products entering Chile or being transported within the country.
- When fields are first used to grow potato plants for the purpose of breeding true potato seed, production sites must have been free from potato crops for ten years prior to planting. Once virus-free stock is initiated, a non-host crop will be grown in the field every two years. If a non-certified potato crop is grown in the field, another ten-year waiting period would ensue.

- Production sites are located at least 200 meters from any other potato crop.
- Fields and their perimeters are regularly scouted for weeds, and any weeds found are eliminated. Pesticide applications to control insect vectors occur throughout the season.
- Plants are tested for the target viruses (PBRSV, APLV, AVB-O, PYV and PVT) during growth at a sampling rate designed to provide 99% confidence of detecting an infection of 1% of plants. If a virus is discovered, all plants within one meter of the infected plant or plants are destroyed and remaining plants are re-tested for the target viruses.

<u>Shipment</u>

- Plantlets arriving in the United States are re-tested using NCM-ELISA and NASH non-reagent tests to confirm the previous testing.

In this Systems Approach, the efficacy of risk mitigation is verified by viral testing. Efficacy is ensured by the eradication of infected plants at each stage of the production scheme.

References

1. Salazar, L.F. "*Potato black ringspot nepovirus*" in: Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 16th Brunt, A.A., *et al* (eds.), January 1997, published online at: http://bioogy.anu.edu.au/Groups/MES/vide

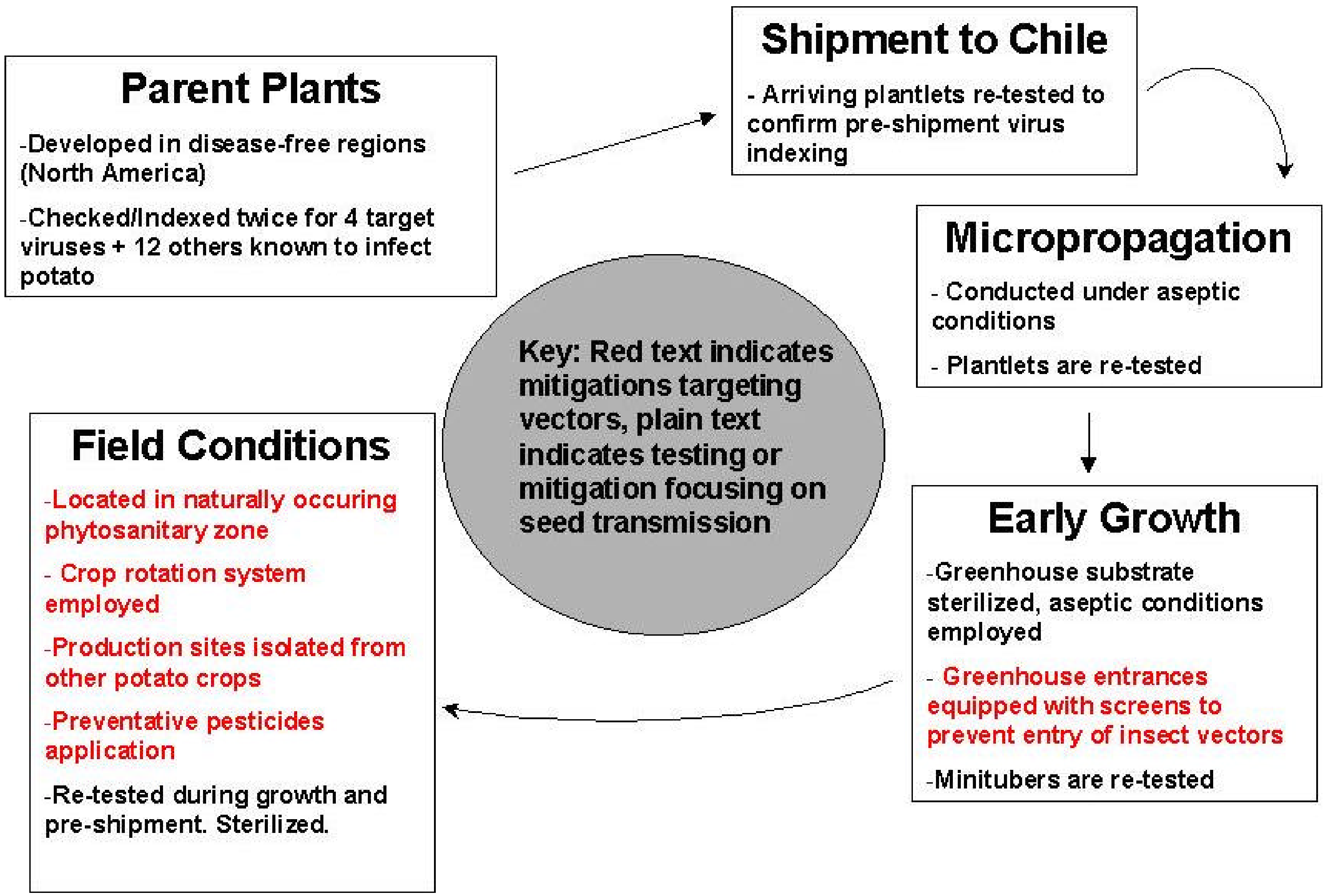
2. CFR 319.37-2 USDA-APHIS Foreign Quarantine Notices

3. Smith, I.M., *et al* (eds.) Data Sheets on quarantine pests for the European Union and for the European and Mediterranean Plant Protection Organization *in*: Quarantine Pests for Europe, Second Edition pp. 1177-1179, 1294-1297, 1302-1304, 1311-1313, 1321-1322. Publication Date.

4. Ministry of Agriculture, Chile. Production of Botanical Potato Seed for Export to the USA, Protocol VII. (Draft document, unpublished). 1995.

Figure 4. Andean Potato Virus Distribution

Figure 5 -- Mitigation steps against potato viruses in production of potato breeding stock in Chile for use in United States



European Stone Fruit Yellows Phytoplasma

European stone fruit yellows is the relatively new name for the phytoplasma in Group X that causes decline of almonds, apricots, peaches and plums in Europe. Previously, this pathogen has been referred to as apricot chlorotic leaf roll, plum leptonecrosis, or European peach yellows. This phytoplasma is the predominant cause of decline and death of productive apricot, peach, and plum trees in Europe.

Distribution

Diseased apricots were first observed in France early in the 20th century. After the discovery of phytoplasmas in 1968, these pathogens were associated with declining stone fruit trees in all Mediterranean countries of Europe and as far north as Germany and recently England. With the development of molecular methods for grouping phytoplasmas and analyzing their nucleic acids, phytoplasmas infecting various stone fruits in these countries were found to be similar. Outside Europe, this pathogen has only been reported in Turkey.

Symptoms

In spring, diseased trees may be easily recognized when leaves emerge prematurely before flowers. Affected leaves are small, yellow, rolled, stiff and brittle. Trees infected when young show leaf symptoms throughout the tree, but old trees may initially have only a few twigs or a scaffold branch affected. Fruit may be small, bumpy, and drop prematurely. As fall approaches, leaves may abscise prematurely. Buds may break producing many weak shoots that are subsequently killed in winter.

Infected trees decline and eventually die within 1-4 years. Over this time, the tree exhibits poor growth, irregular and sparse flowering, few small fruits and discolored phloem. The speed of decline is influenced by the tree’s age, environmental conditions, inherent susceptibility and rootstock type.

Some cultivars show few symptoms before dying the next winter. In general, apricots die more quickly if propagated on peach rootstocks rather than on plum rootstocks. Japanese plums decline more slowly than apricots.

Disease Cycle

This phytoplasma is spread in the field by one or more vectors that have not yet been identified. If this phytoplasma is similar to other stone fruit phytoplasmas, the vector or vectors will probably be phloem-feeding leafhoppers. Trees usually start showing symptoms 1-3 years after inoculation by vectors. Up to 20% of an orchard may be infected in a growing season. New infections occur near diseased trees or along borders of the orchard. In France, wild roses, ash trees, and hackberries in the vicinity of an apricot orchard were found to be infected. Up to 60% of trees in an orchard have been killed within 10-15 years.

International Dissemination

The most important method of long distance dispersal of European stone fruit yellows phytoplasma is by the movement of diseased trees or budwood to infested areas. Since this phytoplasma, like all phytoplasmas, is too small to see, is confined to the phloem tissue, and causes no consistent symptom in dormant wood, the grower or nurseryman is unaware of its presence in transported nursery stock. After planting or propagation, the infected trees serve as a pathogen source for any local vectors that might be present.

This pathogen may be disseminated by the movement of infected wild roses, ash trees, hackberries and perhaps other native host plants. The risk associated with this pathway is difficult to assess due to the lack of essential biological data.

Seed transmission of phytoplasmas is not thought to be possible. So host plant seed~~s~~ are not considered to be a risk for dissemination of this pathogen.

Control

In order to exclude European stone fruit yellows phytoplasma, Prunus hosts from affected countries must be tested in quarantine by grafting tissue collected from candidate plants on to sensitive indicator plants, or by using nucleic acid probes or PCR. Since this pathogen is unevenly distributed in infected budwood or trees, testing must be thorough, as well as accurate. Prunus species produced by approved foreign certification programs can also be imported with reasonable safety.

In Europe, growers must live with this pathogen. Their certification programs can produce trees free of this phytoplasma, but once planted these certified trees are vulnerable to infection by the feeding of vectors carrying the pathogen. Vector populations can be reduced by controlling weeds and leafhoppers in and around the orchard. Tolerant varieties may produce a good crop in severely affected areas. Injection of oxytetracyclines has been successful in suppressing phytoplasma multiplication in infected trees; but, treating a whole orchard is not practical.

Systems Approach

Inspections to detect European stone fruit yellows phytoplasma will not be consistently effective at excluding this pathogen. These single-celled organisms are too small to see, they're buried in the phloem of affected plants, and symptoms in dormant wood are not readily diagnosed using most common methods used at ports-of-entry inspections to be effective. Inspections during growth may detect leaf symptoms when the strain, host, and environment are optimal for symptom expression; but, many suboptimal situations make pathogen introduction inevitable if inspection alone is used to identify and exclude infected plants or budwood.

The only effective methods of exclusion involve testing imported trees or budwood for phytoplasmas. This testing can be used to establish pest-free areas or pest-free production sites. At the present time, the United States does not recognize any pest-free areas in Europe. In the case of pest-free production sites, the United States accepts hosts from government research stations in five countries where trees have been tested and maintained free of this phytoplasma and other pathogens. This restriction reduces the risk of field spread from nearby infected plants that is inherent in importing the same hosts from certified nurseries located in infested countries.

Seeds of hosts are the only plant part that can be safely imported without pathogen testing. While the importation of host plant seed is considered to be safe, importation of seed from commercial cultivars is useless because commercial fruit tree cultivars don't breed true. However, the seeds of host species used to produce rootstock seedlings could be safely imported.

Risk mitigation action initiated in 1980 include:

- Parent stock must be visually inspected, tested and found to be disease free.
- Plants must be inspected and certified as disease free prior to export.
- Plants must pass an inspection upon arrival to the United States.
- Plants must pass through two years of post-entry quarantine prior to being released.

Using the foregoing measures and performing appropriate audits of disease occurrence, the introduction of European stone fruit yellows phytoplasma into the United States following accepted protocols has has been and is likely to be prevented in the future.

References

1. Nemeth, M. 1986. Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees. Martinus Nijhoff, Dordrecht, The Netherlands. 841 pp.

2. Ogawa, J.M., Zehr, E.I., Bird, G.W., Ritchie, D.F., Uriu, K., and Uyemoto, J.K. 1995. Compendium of Stone Fruit Diseases. APS Press, St. Paul, MN.

Monilinia fructigena Honey

Three species of Monilinia cause brown rot of stone fruits. *Monilinia fructicola* and *M. laxa* occur in the United States, but *M. fructigena* does not. *M. fructigena* was reported in Maryland once but was eradicated. In addition to stone fruits, *M. fructigena* also causes damage on pome fruits and other Rosaceous plants.

Distribution

M. fructigena occurs throughout Europe and Asia as well as in parts of Africa and South America. Its discovery in the United States resulted in a program leading to its eradication (Fig. 6).

Symptoms

Under favorable conditions, blossom blight may occur in spring. The fungus spreads into twigs and eventually branches causing twig blight and branch cankers. Gumming may occur at the margins of infected areas. Cankers that girdle branches cause the collapse of distal portions of the branch. Fruit develop firm brown circular spots, especially around injuries, which spread rapidly to envelop the entire fruit. Conidia emerge on the surface of the brown rot. Ripe fruit may develop brown rot lesions during storage.

Disease Cycle

This fungus overwinters in dead tissue, such as fruit mummies, twigs and branch cankers (Fig. 7). In early spring, sporodochia with conidia (asexual stage) develop on fruit mummies, blighted blossoms, or infected twigs and branches. In rare cases, apothecia (sexual stage) may develop from fruit mummies or debris on the orchard floor. Spores are disseminated by wind and rain. Warm temperatures and wet conditions favor spore germination and infections. Insects may facilitate infection by causing injuries or by transporting spores to susceptible tissue. Conidia from blossoms, twigs and branch cankers are the inoculum for fruit infections. Fruit can be infected by direct penetration of the cuticle, stomata or trichomes and through cracks and injuries. Conidia are produced throughout the growing season and can infect fruit in any stage of its development. Decay in storage results from infections just before harvest. Infection of the twigs and branches to form cankers and fruit to form mummies assures fungus survival from season to season.

International Dissemination

The highest risk of long distance dispersal is movement of infected trees or budwood to uninfested areas. Even though large twig cankers may be evident, recent or dormant infections may not be detectable. Once planted or propagated, infected wood will inevitably be exposed to the warm wet conditions necessary for conidia production and the wind and rain necessary for conidia dispersal.

Rotting fruit or recently infected fruit also may carry this brown rot fungus into the United States. However, the usual methods of transportation and storage of fruit minimize the risk of establishment. Rosaceae susceptible to this fungus are quite prevalent throughout the United States, and environmental conditions are favorable for infection during the growing season.

Properly cleaned seed presents a low risk of transmission and spread.

Control

Protective fungicide treatments for both blossoms and fruit are the primary methods of control. These pesticide applications are spaced throughout the growing season to protect fruit up to harvest, minimize storage rot, reduce sporulation, and decrease overwintering inoculum. The removal of fruit mummies and infected twigs or branches and insecticide applications to control vectors and fruit wounding insects also can help to reduce *M. fructigena* damage.

Systems Approach

Inspections to detect *M. fructigena* in dormant budwood, trees or fruit may be successful in finding large cankers or lesions, but it is doubtful whether the smallest and most recent infections will be detected. Various treatments or storage conditions may reduce the risk. Safe disposal of rotten fruit or infected wood is a must. Host plant material imported for use as propagative materials should be held in a quarantine containment facility or field plot well isolated from other host plant materials in the family Rosaceae.

Steps initiated in 1980 to mitigate the risk of spread into the United States include:

- Parent stock must be visually inspected, tested and found to be disease free.
- Plants must be inspected and certified as being disease free prior to export.
- Plants must pass an inspection upon arrival to the United States.
- Plants must pass through a two-year post entry quarantine prior to being released.

Introduction of *M. frutigena* into the United States has been and is likely to be prevented in the future using the foregoing Systems Approach.

References

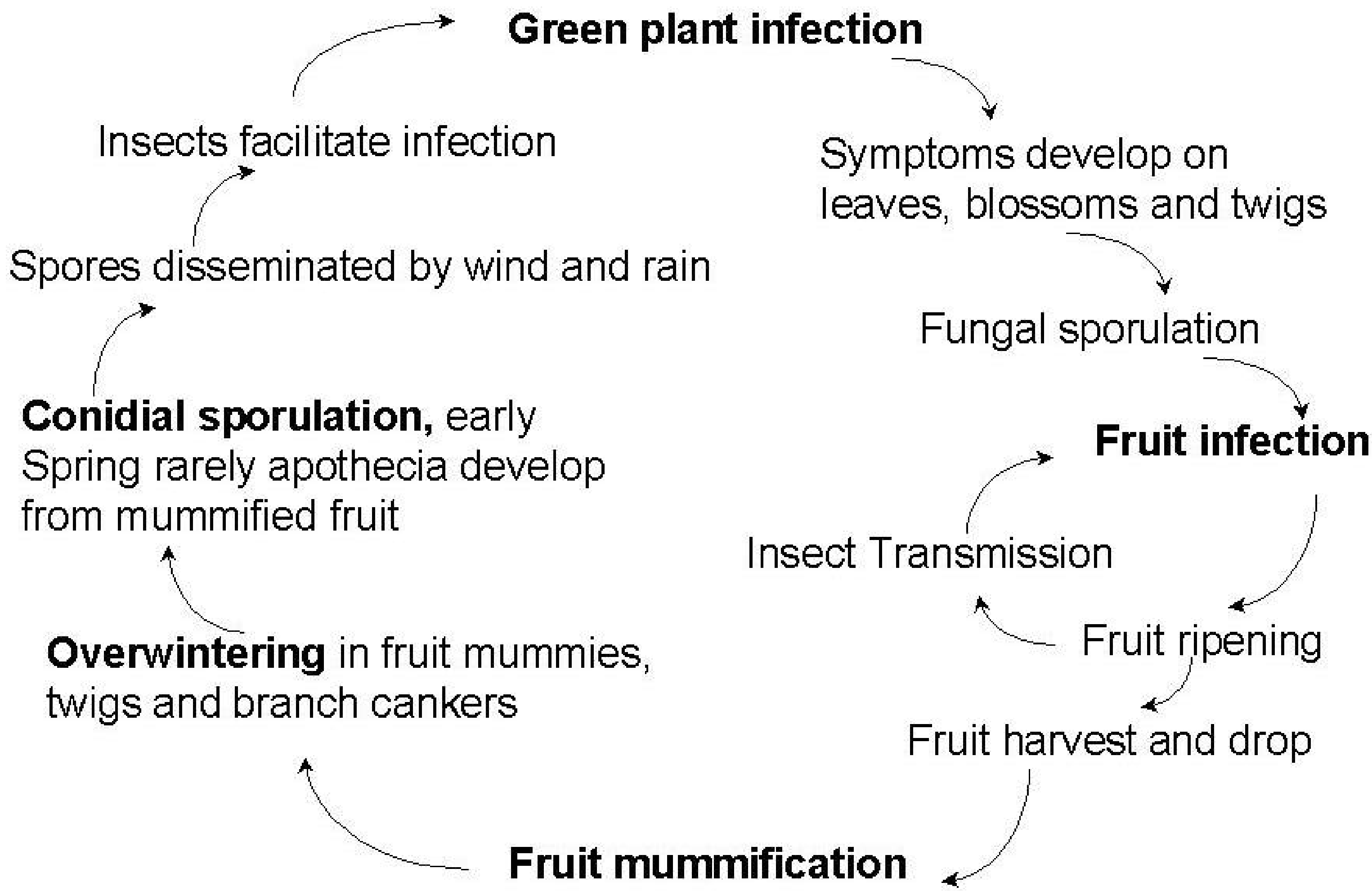
1. CAB INTERNATIONAL. 2000. Crop Protection Compendium. Wallingford, UK: CAB INTERNATIONAL.

2. Chang, L.W.H. 1986. A fruit brown rot. PNKTO No. 76. USDA-APHIS, 14 pp.

3. Ogawa, J.M., Zehr, E.I., Bird, G.W., Ritchie, D.F., Uriu, K., and Uyemoto, J.K. 1995. Compendium of Stone Fruit Diseases. APS Press, St. Paul, MN.

Figure 6. Brown Rot (*Monilinia fructigena*) Distribution
(**CAB International 2000**)

Figure 7. Disease cycle of brown rot caused by *Monilinia fructigena*.



Plum Pox Virus

Plum pox virus (PPV), also called sharka, is the most damaging pathogen of stone fruits in Europe. Serious losses in European plum, Japanese plum, peach, nectarine and apricot are common in central and eastern Europe. Occasionally almond may be infected. Sour cherry and sweet cherry have been infected by unique PPV strains in southeastern Europe. The most common strains, the D and M strains, are not known to infect cherries. Serious losses have only been documented for the D and M strains of this virus.

Distribution

PPV was first observed around 1918 in Bulgaria. During the next 50-60 years, the virus was spread throughout Europe except for certain areas of Scandinavia. More recently, the virus has been found in Turkey, Syria, Egypt, Chile, India, the United States (PA), and Canada (Ontario) (Fig. 8).

Plum pox is being eradicated in the United States. In Canada, the Canadian Food Inspection Agency has negotiated a three-year program with the industry and other stakeholders. On the Niagara peninsula, the effort is being characterized as "rapid containment". Unlike in the United States, the only trees that are being removed are in groups of four – as represented by positive, four-tree samples.

Symptoms

Symptoms vary with the cultivar, virus strain and environment. In some situations, cultivars may exhibit leaf and fruit symptoms; other cultivars may have only leaf symptoms and others only fruit symptoms.

Symptoms may begin in young leaves as veinclearing and develop into chlorotic spots, rings or lines during spring and early summer. In some cultivars, chlorotic symptoms may turn necrotic. In others, chlorosis may be transient and fade in the summer heat. Twisting and leaf distortion may occur in peach leaves. Symptoms may be localized or scattered in the tree, due to uneven virus distribution.

Fruit symptoms appear as chlorotic rings or blotches in unripened fruit. Necrosis may develop in some varieties. In other cases, fruit may become deformed with bulges and irregular grooves and depressions. Red rings or spots may occur on the stones of affected fruit. Fruit may be fibrous and lack flavor. Fruit of some cultivars may drop prematurely causing total crop loss.

Disease Cycle

Plum pox is a potyvirus that is spread locally by many aphid species (Fig. 9). The virus does not persist in an aphid vector after it has been acquired from an infected source. An aphid vector may acquire the virus by merely probing infected tissue. Actual feeding or colonization of infected trees is not required. Known vectors are widely distributed throughout the United States wherever stone fruits are grown. Infection results when an aphid vector probes an infected leaf, acquires the virus, and then probes a healthy leaf within seconds or minutes after acquisition. Protective pesticides have not been effective at preventing infection because transmission occurs quickly upon probing of host plant tissue by a viruliferous aphid. However, under certain circumstances, insecticides can slow virus spread by reducing vector populations.

After transmission, virus spread within a healthy tree may be slow and erratic. Initially, a few areas on one or two limbs may be infected and only a few of these may show leaf symptoms. Aphids may acquire and further spread the virus from a tree before the tree begins to show symptoms or is determined to be infected by testing. In fact, PPV may not become systemic and show obvious symptoms in a large tree until years after the initial inoculation.

Some studies suggest that optimal transmission occurs in May-June and in September-October, but so many aphid species are known to be vectors that transmission at some level may occur throughout the growing season.

Viruliferous aphids will transmit PPV to any nearby hosts, whether they are commercial cultivars, ornamentals or wild Prunus species. With strains in the D group from western Europe, the natural host range seems to be confined to Prunus species. However, reports from eastern Europe suggest that walnut, ornamentals, and some weeds may become naturally infected with strains from these localities.

International Dissemination

Long distance dispersal of PPV is by the movement of diseased trees or budwood to uninfested areas. Since this virus is too small to be observed with the unaided eye or a conventional microscope and causes no consistent symptom in dormant propagating material, the commercial grower or nurseryman is unaware of the presence of PPV in his or her nursery stock. Once infected trees are planted or propagated in a new location, local aphids begin spreading PPV as soon as new infected leaves emerge.

Seed transmission, thought to be a second means of long distance spread by some, is controversial. Many studies in western Europe with D strains and local cultivars failed to detect seed transmission of PPV, but some research in eastern Europe showed seed transmission at low levels with their strains and cultivars.

Control

Many countries that are free of PPV try to prevent its entry. Prunus species imported from infested countries are held in secure post-entry quarantine facilities and tested using-sensitive indicator plants, ELISA serology, or RT-PCR. Foreign country fruit certification programs with a demonstrated capacity for excluding the plum pox virus from stone fruit planting and propagative stock are a possible source of clean stock for safe importation.

In some countries, PPV is confined to certain areas by containment and/or suppression programs. The French have been successful in confining PPV to southeastern France, and the Dutch attempt to eradicate the virus whenever they find it. Unfortunately, none of the European countries have been successful in eradicating PPV entirely from their country for sustained periods of time.

Countries where PPV is widespread are trying to live with it. Their stone fruit tree certification programs can produce PPV-free trees; but, aphids carrying the virus from nearby infected trees eventually infect certified trees planted in infested areas. Plum, peach and apricot crops of reasonable quality are only possible using tolerant varieties. Transgenic virus-resistant stone fruits have shown promise under these conditions.

Systems Approach

Inspections to detect the presence of PPV will not be consistently effective at excluding this pathogen. The virus is too small and symptoms in dormant wood are too rare for inspections at ports of entry to be effective. Inspection during the growing season may detect foliar and fruit symptoms when the strain, host and environment are optimal for symptom expression. Suboptimal combinations make PPV introduction inevitable where visual inspection and exclusion from shipment are used as the sole risk mitigation measure.

The only effective methods of exclusion involve the use of virus testing methods. Testing can be used to establish pest-free areas or pest-free production sites. For pest-free areas, the foreign plant protection organization must demonstrate by acceptable surveys that PPV does not occur in the designated area and will not be introduced into that area because of an effective quarantine program. Monitoring surveys over time will also be needed. The surveys and quarantine programs must involve testing trees for PPV. For pest-free production sites, the foreign plant protection service must have an acceptable certification program which involves testing mother plants for PPV and controlled propagation from these tested trees in a manner that minimizes the probability of PPV spread to these certified trees before export.

The United States currently accepts PPV hosts only from government research stations in five European countries where the trees have been tested and maintained free of PPV. Limiting importation to host materials originating from government research stations reduces the risk that is inherent in importing the same hosts from producing stock in an infested area or country. Plants imported from government research stations are inspected and must also go through a two-year post entry quarantine before their release. Cherries in the subgenus Cerasus can be imported in commercial quantities from nurseries in approved certification programs in these five countries.

References

1. CAB INTERNATIONAL. 2000. Crop Protection Compendium. Wallingford, UK: CAB INTERNATIONAL.

2. Nemeth, M. 1986. Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees.

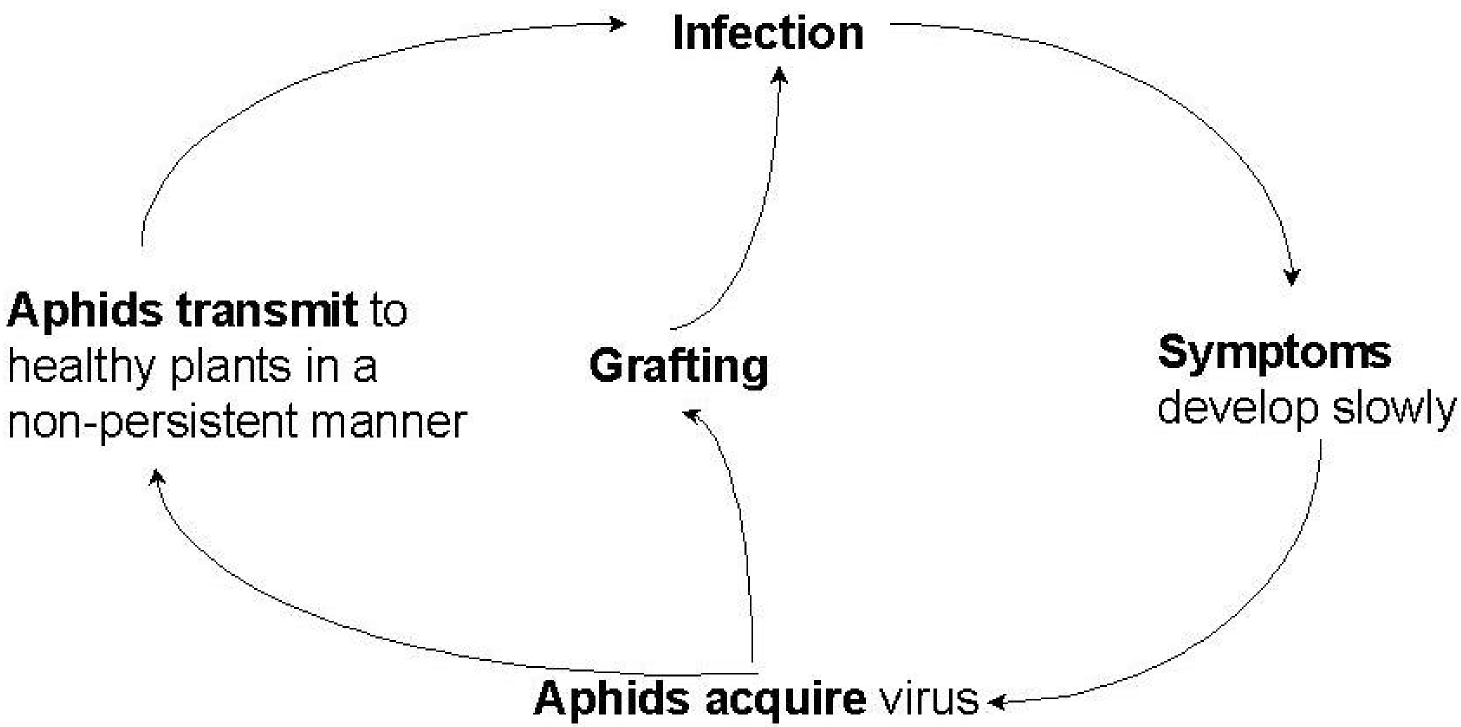
Martinus Nijhoff, Dordrecht, The Netherlands. 841 pp.

3. Ogawa, J.M., Zehr, E.I., Bird, G.W., Ritchie, D.F., Uriu, K., and Uyemoto, J.K.

1995. Compendium of Stone Fruit Diseases. APS Press, St. Paul, MN.

Figure 8. Plum Pox Distribution (CAB International 2000)

Figure 9. Disease cycle of plum pox virus



Chrysanthemum White Rust

White rust of chrysanthemums is caused by the fungus *Puccinia horiana*. Chrysanthemums are the only known host of *P. horiana* . The chrysanthemum genera most susceptible include Dendranthema, Nipponanthenium, Leucanthemella and Ajania. Leaves, stem and inflorescence can all be affected.

Distribution

White rust was first identified from Japan in 1895. Its subsequent distribution appears to have been to China and then South Africa. Infestations into Europe came from both Asia and Africa. Australia and New Zealand infections are of fairly recent origin with the infested areas of Southern Australia, Tasmania and Western Australia being under active control. *Puccinia horiana* was introduced repeatedly into the Western Hemisphere during the last three decades. Its current distribution includes every arable continent (Fig. 10).

North America has actively initiated eradication and exclusion programs to prevent further introductions and spread of the pathogen. In the United States, a control program focused on preventing the disease in commercial production operations has been implemented.

Symptoms

Following infection with *P. horiana*, the upper surfaces of chrysanthemum leaves exhibit small pale green–yellow–tan spots up to 5 mm in diameter. The corresponding areas on the lower leaves are initially pinkish-white. As the spots enlarge, raised pinkish pustules are seen which change to white with the production of basidiospores as they mature. With severe infections sori sometimes develop on bracts and stems. Florets occasionally exhibit necrotic flecking with pustules.

Disease Cycle

Puccinia horiana is an autoecious rust. Following conditions of high humidity coupled with a film of moisture, with a temperature range of 4–23C with an optimum of 17C, bicellular teliospores germinate to produce unicellular basidiospores, which are dispersed into the air. Air currents distribute the spores to chrysanthemums to initiate new infections. For infection to occur, conditions of high humidity with a film of moisture for a minimum of 5 hours are needed. While the temperature range for infection by basidiospores to occur is 17-24C, no optimum temperature has been noted. Basidiospores are very sensitive to desiccation at a relative humidity below 90%. The incubation period from infection to initial symptoms is usually 7–10 days but short periods of high temperatures can prolong this period for up to 8 weeks. Teliospore production is initiated a few days following the development of symptoms. (Fig. 11)

International Dissemination

Even though basidiospores dispersal is known to occur over distances of 700 m, long range spread of the disease would not be likely because of their sensitivity to desiccation. White rust is more commonly spread on infected cuttings and plants from greenhouse grown chrysanthemums and cut flowers as they are distributed around the world.

Control

Preventive spraying with fungicides control the disease but is not cost effective. Biological control using *Verticillium lecanii* has shown some promise in greenhouses. Breeding for resistance is ongoing with numerous resistant varieties available. However, a variety resistant to one strain of the fungus may be susceptible to a different strain. Exclusion and eradication strategies are commonly employed to control the disease.

Systems Approach

A Systems Approach to circumvent further introduction of *P. horiana* into the United States was began in 1980. The specific risk mitigation measures include:

- Parent stock must be visually inspected, tested and found to be disease free.
- Plants must be inspected and tested once a month for 4 months prior to export.
- Plants must pass an inspection upon arrival to the United States.
- Plants must pass through 6 months of post entry quarantine after entry into the United States. (Inspections confirming the material's disease-free state continue for 8 times the normal disease cycle time from infection through sporulation before importation of plants into the United States and continue through 12 times the normal disease cycle time from infection through sporulation during the post entry quarantine period.)

The introduction of white rust into the United States using the foregoing Systems Approach is not likely.

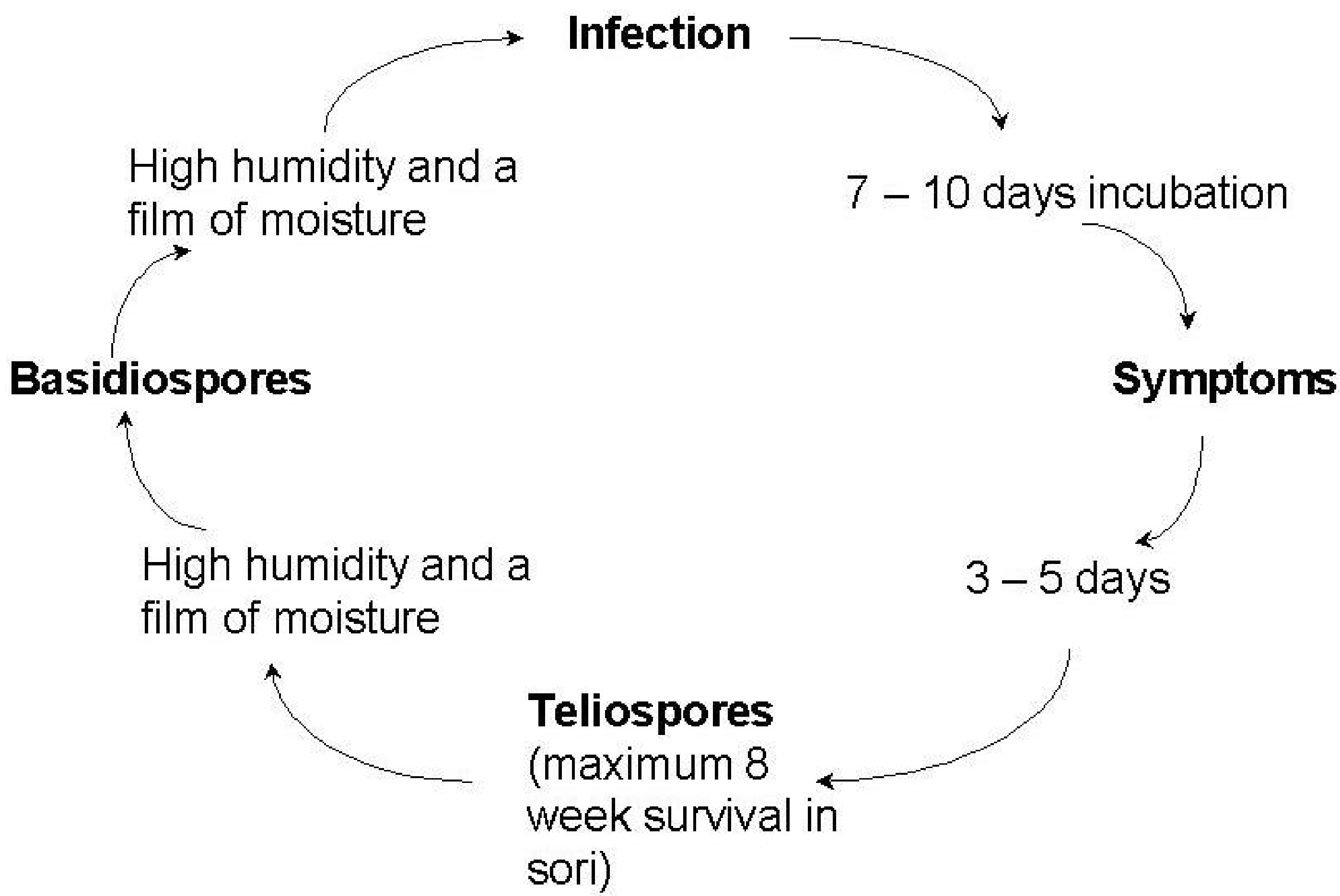
References

1. CAB INTERNATIONAL. 2000. Crop Protection Compendium. Wallingford, UK: CAB INTERNATIONAL.

2. Horst, R.K. and Nelson, P.E. 1997. Compendium of Chrysanthemum Diseases. APS Press, St. Paul, MN.

Figure 10. Chrysanthemum White Rust Distribution
(CAB International 2000)

Figure 11. Disease cycle of chrysanthemum rust caused by *Puccinia horiana*.



A Case Study In Systems approach For Citrus Canker: Unshu Oranges From Japan And Korea – Argentine Citrus

Citrus canker is caused by a bacterium, *Xanthomonas campestris* pv. *citri*. Strains of the bacterium vary in virulence and aggressiveness with respect to host, cultural and physiological characteristics, bacteriophage sensitivity, serology, DNA-DNA homology, and genomic fingerprinting (e.g., RFLP and AFLP analyses). This pathogen is believed to have originated in Asia where the most aggressive (Group A) strains occur.

Group A (Asiatic canker), group B (cancrosis B), group C (Mexican lime cancrosis), and group D (citrus bacteriosis) are distinguished on the basis of host specificity and pathogen aggressiveness. The restricted host ranges and weak pathogenicity of group B, group C and group D strains is widely recognized.

Citrus canker affects the economy of countries that have the disease as well as those countries that do not. It results in:

- Reduced marketability due to blemished fruit.
- Reduced fruit yield (e.g., premature fruit drop) of susceptible species/varieties.
- Reduced tree vigor (e.g., terminal dieback, defoliation) of susceptible species/varieties.

Increased costs to consumers/taxpayers to implement additional disease-specific phytosanitary measures (e.g., eradication program, regulation of movement of fruit and/or plant propagating material, additional protective bactericidal/bacteriostatic sprays).

Loss or restriction of market development/maintenance/enhancement from areas where the pathogen is introduced and becomes established, and/or where the disease is endemic.

Distribution

The distribution of *X. campestris* pv. *citri* is shown in Figure 12. A complete listing of occurrences can be found in CAB International 2000.

The geographical distribution of *X. campestris* pv. *citri* differs for different types of citrus canker. Canker A (Asiatic canker) is found in Asia, South America, Oceania and the United States. This strain is the predominant and potentially the only strain still found in nature. While canker B (Cancrosis B) has been reported in South America, canker C from Brazil and canker D from Mexico, neither they nor the diseases they cause are found in the field anymore.

X. campestris pv. *citri* has been eradicated from Australia including Thursday Island and South Africa.

Symptoms

Fruit lesions vary in size because the rind is susceptible for a longer time than the leaves and are subject to multiple infection cycles. Fruit and stem lesions are up to 1 mm deep and superficially resemble those formed on the leaves.

Following infection, symptom development depends upon host, plant part and age of tissue, and environmental conditions at the time of infection. On leaves, lesions start as small circular spots 2-10 mm in diameter. As the leaf lesions age, they may become irregular. Since leaves are only susceptible for a short period of time, infection is usually restricted to a single occurrence. Therefore, lesions tend to be about the same size and tend to aggregate at the leaf margins, leaf tip, or on a restricted area on the leaf. Infections can be increased by injury resulting from wind-caused abrasion and feeding of the Asian leaf miner (*Phyllocnistis citrella*) to the point that field resistance and tolerance are negated.

Lesions develop as light yellow, raised, spongy eruptions on the surface of leaves, twigs and fruits. As the lesions enlarge, the spongy eruptions begin to collapse, and brown depressions appear in their central portion, forming a crater-like appearance. The edges of the lesions remain raised above the surface of host tissue and are characterized by having a greasy appearance. As the disease advances, the central portions become grayish-white, hard and appear as corky dead tissue with a rough surface surrounded by yellow halos. Canker lesions retain the erupted and spongy appearance under dry conditions; whereas they quickly enlarge and turn to flat lesions with a water-soaked appearance with frequent rain. The lesions of canker groups B, C and D are generally similar in appearance and histology to those of canker A, but they are significantly smaller.

The darker developing lesions on lemons and limes together with the water-soaked margin that develops around the necrotic tissue, which is easily viewed with transmitted light, are useful diagnostic symptoms for canker.

Disease Cycle

X. campestris pv. *citri* survives in lesions in leaves, stems and fruits. (Fig. 13) It can also survive on woody branches for several years. During periods of free moisture on the lesions, the bacteria ooze out and can be dispersed to infect the receptive host tissue. Wind-driven rain is the primary mode of dispersal. Winds in excess of 8 mph contribute to successful penetration of the bacteria through the stomatal pores or wounds made by insects, blowing sand or thorns. Injury resulting from pruning followed by environmental conditions favorable to the dispersal of the causal bacteria can result in severe infections.

X. campestris pv. *citri* continues to multiply while the lesion is expanding. The number of bacteria produced is dependent upon the susceptibility of the host and tissue infected. The bacteria remain viable in the margins of leaf lesions and fruit until they fall. Exposed bacteria that have oozed onto the leaf surface begin to die when conditions are dry. Exposure to direct sunlight accelerates bacterial death. Survival of exposed bacteria is only a few days if in the soil and only a few months in infected plant debris that is decomposing in the soil. However, the bacteria can remain viable for years in infected plant debris that is dry and free of soil.

Epidemiology

Host resistance to infections increases as the plant tissues age. Nearly all infections occur on the leaves and stems during the first 6 weeks of growth within a season. The fruit rind is most vulnerable during the first 90 days after petal fall. Fruit and foliage resistance is directly related to cuticle formation. As the cuticle thickens, resistance increases.

Environmental conditions delaying tissue maturation or promoting new shoot emergence favor disease development. Most spread of the bacteria is over short distances, i.e., within a tree canopy or to neighboring trees. While some severe meteorological events can contribute somewhat to relatively short distance spread of the pathogen with concomitant increase in disease incidence, long distance dispersal of the pathogen is largely through the movement of diseased propagating material such as budwood, rootstock seedlings or budded trees. There is no record of seed transmission of *X. campestris* pv. *citri*. Dispersal can occur from nursery workers carrying the bacteria on their person and equipment unless properly disinfested. Long distance spread of the pathogen can also occur if infected cull fruit are deposited near citrus orchards. Infected commercial fruit also can result in long distance spread when it is transported to uninfested areas and subsequent handling results in the transfer of bacteria to citrus trees. Boxes carrying infected fruit also have been implicated in long distance dispersal.

Control

Exclusion is the first line of defense against citrus canker. Much of the credit for the fact that citrus canker does not occur in all citrus production areas where environmental conditions are conducive to development of the disease is due to restrictions on the importation of propagating materials and fruit from infected areas. If new canker infestations in a previously uninfested area are detected before they are well established and widespread, removal and destruction of the infected trees and their uninfected, but exposed neighbors is an accepted form of eradication.

In the early 1900’s, canker was reported in South Africa, Australia and the United States (Gulf States only) but was eliminated through the implementation of orchard inspections, quarantines, and on-site removal and destruction of infected trees. Eradication programs are ongoing in Florida where new infections are found.

Integrated disease management is employed in areas where canker is a major problem. Integrated management includes the use of windbreaks, leafminer control, applications of copper sprays and the use of resistant varieties.

Systems Approach

Development and implementation of Systems Approach has effectively prevented the introduction of the citrus canker pathogen into the United States from Japan and Korea. Integrated pest management actions lend themselves to the Systems Approach concept. The establishment of disease free groves or production areas followed by the application of additional risk mitigation measures reduces the risk of artificial spread from these infested countries to a miniscule level.

In the case of United States importation of Unshu oranges from citrus export regions in Japan and Korea, host susceptibility is an important, fundamental consideration. Unshu orange (*Citrus unshui*) resistance to *Xanthomonas campestris* pv. *citri* infection is moderate to high. It is the only species permitted to be grown for production in citrus export regions. These practices are incorporated as part of the following Systems Approach, which help prevent the artificial spread of the pathogen to the United States (Figure 14):

- Unshu oranges must be grown in a Citrus Canker-free production area.
- A 400-meter buffer zone must be maintained around the canker free area.

- Only Citrus Canker-resistant species can be grown in the buffer zone.
- Visual inspections for Citrus Canker are conducted in the groves at packing to verify freedom from disease.
- All fruit must be surface sterilized before packing.
- All tissue paper wrappings and shipping boxes must be labeled with the distribution requirements.
- The fruit must be inspected to ensure that all requirements have been met prior to its shipment to the United States.
- The fruit may be distributed only to non-citrus producing states or territories.

In the case of citrus shipped from Argentina to the United States, the pathogens targeted include *X. campestris* pv. *citri*, the cause of citrus canker; *Elsinoe australis*, the cause of sweet orange scab; and *Guignardia citricarpa*, the cause of citrus black spot.

The following mitigating steps, as well as stepwise spatial restriction of the imported fruit initially into non-citrus producing areas, were implemented during 2000 (Fig. 15):

- Citrus must be grown in a registered Citrus Canker-free production area to be eligible for export.
- A 150-meter buffer zone must be maintained around the canker free area.
- Fallen leaves, twigs and fruit must be removed from the grove prior to each season's bloom.
- Visual inspections for Citrus Canker in groves are routinely conducted.
- Two copper-oil treatments are applied during fruit formation.
- All blemished fruit are culled.
- Twenty days prior to harvest, fruits are sampled and incubated to check for symptoms.
- Packing house inspection of fruit follows 4-5 days of incubation.
- All fruit is surface sterilized before packing.
- Identity of the fruit's origin, certification and shipping for export must be preserved.
- Separate packing houses must be maintained and used exclusively for export.
- Point of entry inspection is required when the fruit arrives in the United States.
- Fruit may be distributed only to states or territories as deemed appropriate by the statutes during the years the fruit is imported.

This Systems Approach together with surveillance of disease outbreaks makes the artificial spread of citrus canker, sweet orange scab, and citrus black spot into the United States from Argentina highly unlikely.

References

1. CAB INTERNATIONAL. 2000. Crop Protection Compendium. Wallingford, UK: CAB INTERNATIONAL.

2. Civerolo, E.L. 1985. Comparative characteristics of *Xanthomonas campestris* pv. *citri* variants. In: Abstracts on the genus Xanthomonas. Fallen Leaf Lake Conference, Fallen Leaf Lake, South Lake Tahoe, California, September 20-23, 1985, 24 pp.

3. Schaad, N.W., Vidaver, A.K., Lacy, G.H., Rudolph, K., and Jones, J.B. 2000. Evaluation of proposed amended names of several Psuedomonads and Xanthomonads and recommendations. Phytopathology 90:208-213.

4. Timmer, L.W., Garnsey, S.M., and Graham, J.H. Compendium of Citrus Diseases. 2nd ed. APS Press, St. Paul, MN.

Figure 12 CITRUS CANKER DISTRIBUTION

Figure 13. Disease Cycle Of Citrus Canker Caused by *Xanthomonas campestris* pv. *citri*

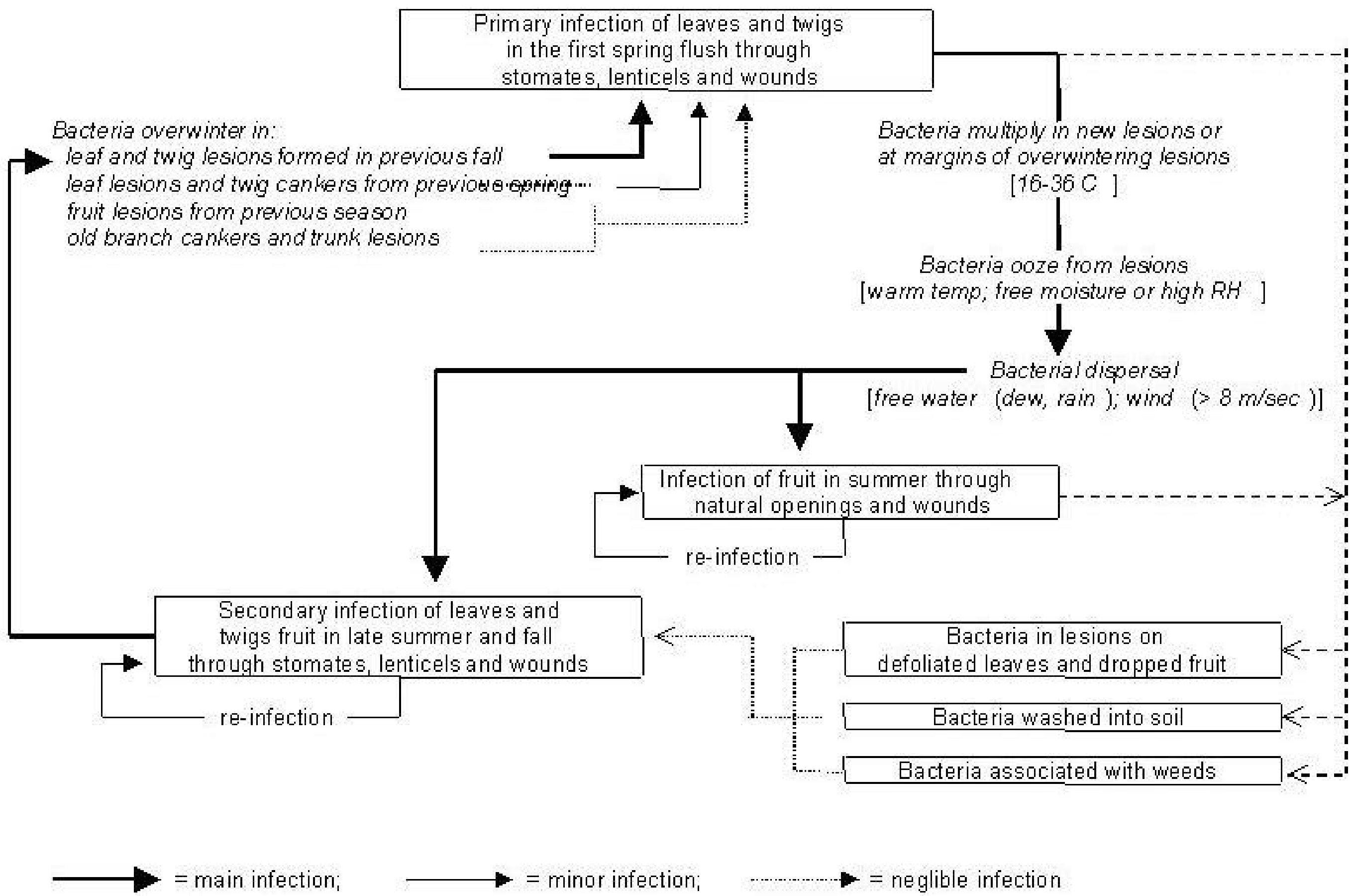


Figure 14. Mitigations in Disease Cycle of Citrus Canker For Unshu Oranges from Japan and Korea

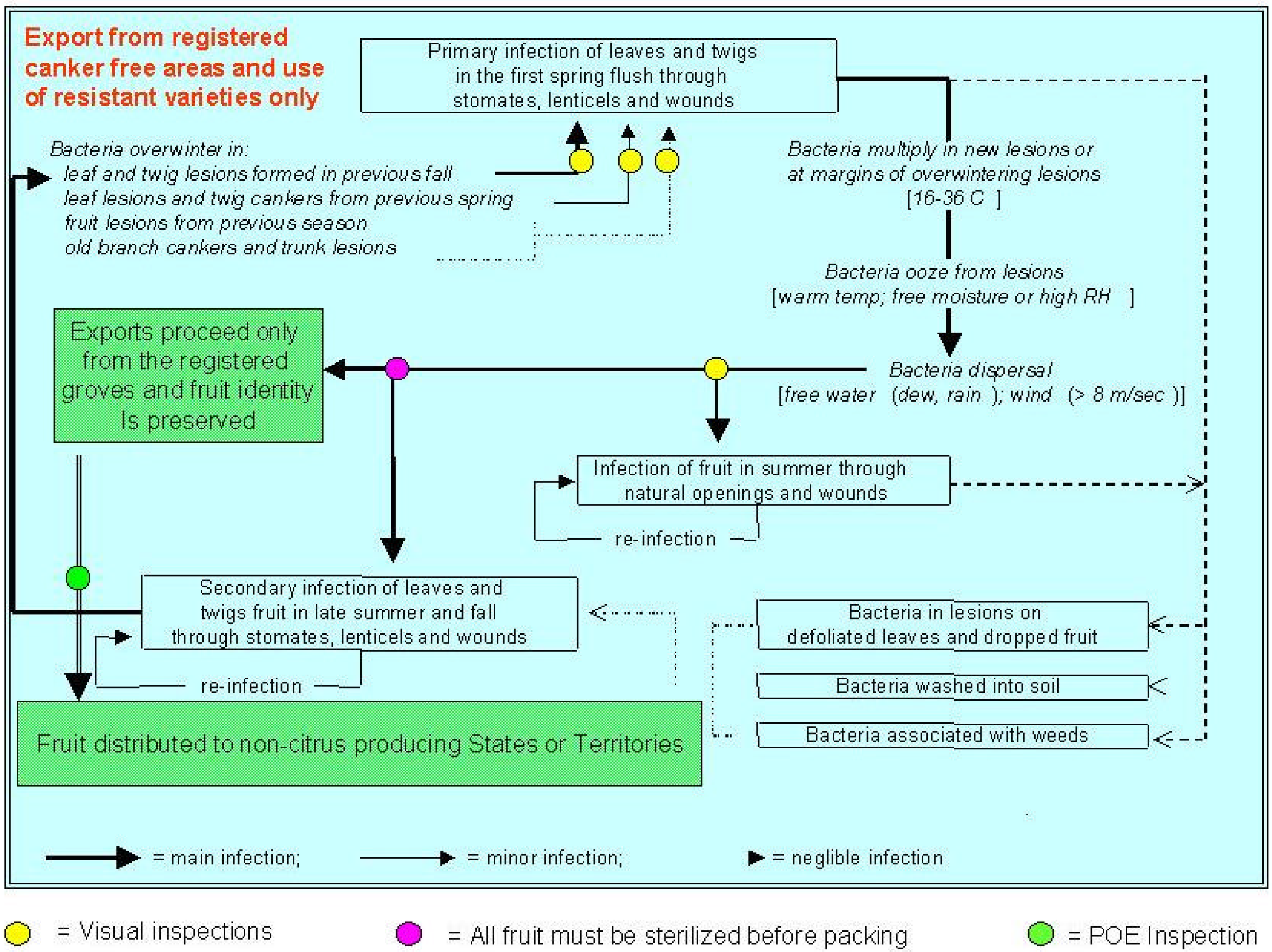
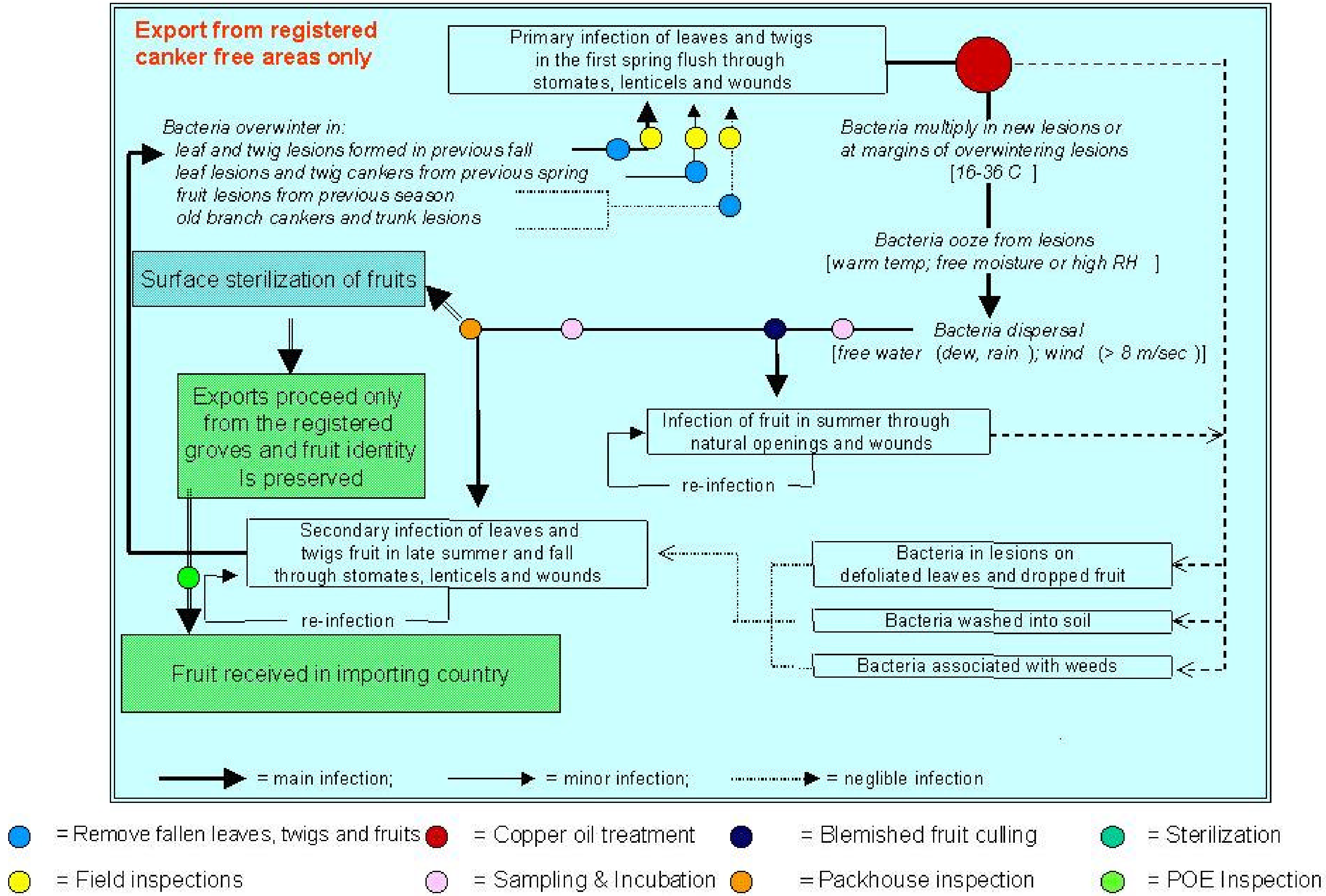


Figure 15. Mitigations Enacted for Argentine Citrus



XI. Available, Alternative Risk Mitigation Methods

Risk mitigation methods available for integration as part of a Systems Approach are numerous. Although available as alternatives some also can serve as “stand alone” measures – depending primarily on the desired level of quarantine security. In general, these alternatives are most useful in situations where there is only one pathogen or disease of concern. Where two or more pathogens/diseases of concern occur, multiple measures often will be required to achieve a particular or specified level of quarantine security.

Some pest risk mitigation alternatives include the following:

Pest-Free Area

More commonly used when the concern is primarily insect pests, this concept has also been employed for plant diseases. These are areas covered by a quarantine or other appropriate exclusion measures to prevent entry and establishment of the targeted quarantine pathogen and wherein freedom from the disease has been and continues to be verified by valid survey. Pest free areas may be large geographic areas or small production facilities such as greenhouses. The key is the ability to ensure that they are and remain pathogen free.

Examples include:

1) Argentine citrus fruit moving from citrus canker-free states to the United States,
2) Florida citrus fruit moving from citrus canker-free production areas to other citrus producing states,
3) South African citrus fruit moving from black spot-free production areas to the United States,
4) Australian citrus fruit moving from black spot-free production areas to the United States, and
5) The Mexicali Valley of northern Mexico as a Karnal bunt-free area, allowing the export of wheat without restriction.

Varietal Resistance

Certain varieties of a given commodity may be resistant, very highly resistant, or even immune to infection by quarantine pathogen, and could be allowed movement on that basis. As an example, certain varieties of ornamental barberries are resistant to infection by *Puccinia graminis* (black stem rust of wheat). Barberry is the alternate (aecial) host of this rust, and is prohibited in some United States locations by federal domestic quarantines. Recognition of the highly resistant status of certain varieties in recent years has allowed them to move into areas of the United States where they historically were prohibited.

Commodity Treatment

Certain commodity treatments are recognized as eradicative for several pathogens of quarantine concern. For example, hot water dips of dormant grape cuttings for precise times at carefully controlled temperatures are known to kill the phytoplasma that causes flavescence doree and the bacterium (*Xylella fastidiosa*) that causes Pierce's disease. These treatments may also be used to eliminate nematodes from certain bare-root plants.

Tissue Culture

Tissue culture alone is not a pest risk mitigation measure. Tissue culture used together with other methodology is a tool for development of pest free stock that can subsequently be multiplied rapidly. When other methodology is included, the result really is an example of a Systems Approach.

Tissue excised from an actively elongating area such as a meristem often will be free of systemic plant pathogens. Such tissue manipulated to grow into plantlets in aseptic culture can be disease tested using available methodologies. If determined to be free of a targeted quarantine pathogen, millions of progeny plants can be produced, first as plantlets in aseptic culture and then to the desired salable size.

If parent stocks are periodically audited (tested) and subsequent production is under conditions that ensure pathogen exclusion, a system is created whereby the artificial spread of quarantine pathogens can be prevented.

Commercial Part of Commodity Free of Quarantine Pathogen

Not all plant parts are infected by certain pathogens, enabling movement of ~~those~~ uninfected parts, even when the disease of concern is known to occur in the production area. An example is *Xylella fastidiosa*, the causal agent of Pierce's disease of grapes. Inasmuch as the causal bacterium does not infect the fruit, grapes can be moved with a high level of quarantine security from a Pierce's disease infested area to one that is not infested.

Arguably, the best-known example is the movement of many different types of seeds~~;~~. Even though pathogens of concern may infect the parent plants, most do not infect the seed.

Growing Season Disease Control

Use of available, effective growing season treatments can prevent infection/infestation of a crop. Inspection prior to harvest and shipping to confirm often is used to complement growing season treatments. As an example, chrysanthemums are produced in greenhouses in Columbia utilizing a carefully timed spray program to control chrysanthemum white rust, which occurs in the production area. The plants then are carefully inspected prior to harvest and shipping to ensure freedom from the disease.

Limited Distribution of Hosts

Usually coupled with other control measures, limiting the region(s) of the importing country where a commodity may be distributed may provide adequate assurance that a disease/pathogen of concern will not be introduced into areas of the United States where the commodity is grown. As an example, several years ago Florida citrus fruit coming from areas where there was uncertainty regarding freedom from citrus canker was limited to sales in the northeastern states, well away from other citrus-producing states in the United States.

XII. Conclusions

In the beginning, concern about the possibility of a plant pathogen of quarantine significance being introduced on a given commodity from another country was sufficient cause to prohibit import of that commodity from that country. The underlying philosophy was "when in doubt, keep it out." Generally, the commodity was prohibited from all regions of a country where the pathogen was known to occur. As an example, imports of citrus fruit from Australia and South Africa were prohibited due to the presence of the black spot, caused by the fungus *Guignardia citricarpa*.

Several years ago, both Australia and South Africa conducted extensive surveys and laboratory analyses of samples to establish that certain large, isolated production areas were free of black spot. Regulations were implemented to prohibit importation of infected nursery stock to these areas. Ongoing surveys were established. Based on the documented evidence presented, APHIS decided to allow the export of citrus fruit from those production areas to the United States.

In recent years, acronyms such as NAFTA, GATT, and WTO-SPS have become a part of the agricultural trade vocabulary. These agreements are paving the way for growth in international trade and – consistent with established disciplines, principles and standards – increased movement of plants and plant products among countries. Increasing political pressures have forced national plant protection organizations to retreat from longstanding, conservative approaches in favor of a "figure out how to do it" approach. The Systems Approach thus serves as an additional, important tool in the United States' efforts safeguard its agriculture, natural resources, and economic well being while facilitating essential trade.

Understanding the scope and effectiveness of available pest risk mitigation methods for a pathogen of quarantine concern is essential to the Systems Approach. Many single treatment pest risk mitigation methods that provided a Probit 9 level of quarantine security have been lost. Others soon will be lost. And new ones will be slow in coming, if ever. The challenge now is to take those still available pest risk mitigation measures and combine them in such a way as to achieve an acceptable level of pest risk for imported host commodities. When a single control method used alone will not ensure achievement of an acceptable level of quarantine pest risk, the application of independent treatments with additive effects is required on a commodity-by-commodity and pathogen-by-pathogen basis. Each commodity/pathogen/country situation is unique. There is no "how to do it" manual.

In many cases, the information needed to enable development of an adequate Systems Approach for a particular commodity/pathogen combination will be quite challenging. At times, it will be impossible. In those cases where there is a considerable level of uncertainty about the efficacy of a Systems Approach (a particular combination of pest risk mitigation methods), a degree of redundancy or "over-kill" might be required to ensure an acceptable level of quarantine pest security.

There is, however, solid scientific evidence for the soundness of the use of the Systems approach to guard against the introduction of plant pathogens into the United States associated with the importation of plants or plant products into the United States. Though the term, Systems Approach, is relatively new, the combining or integration of different pest risk mitigation methods to achieve an acceptable level of quarantine pest security has been used since 1967. While the Systems Approach is scientifically sound, it must be remembered that there is no such thing as "zero risk." Pest risk mitigation methods that provide a Probit 9 level of quarantine pest security are not perfect. The level of protection is not complete and any method is subject to failure when not properly applied.

In addition, as with the application of any method, monitoring and verification are essential elements. Monitoring and verification must be maintained in order to detect changes in the pathosystem and to maintain stakeholder confidence. These essential elements also enable continuous improvement through the discovery and correction of error.

References

1. Burnett, W. 2001. APHIS' Use of Systems approaches as They Relate to Plant Pathogens. Report submitted to study committee May 30, 2001.

2. Couey, H.M. and V. Chew. 1986. Confidence limits and sample size in quarantine research. J. Econ. Entomol. 79:887-890.

3. Finney, D.J. 1971. Probit Analysis, 3rd Ed. Cambridge University Press.

IPPC. 1999. Systems approaches for Pest Risk Management. IPPC Secretariat Discussion Paper. January 1999.

4. IPPC. 2000. Integrated Measures for Pest Risk Management – Systems approaches. Work Group Draft. 27 July 2000. Brisbane, Australia.

5. Jang E.B. and H.R. Moffitt. 1994. Systems approaches to achieving quarantine security. In Quarantine Treatments for Pests of Food Plants, Sharp, J.L. and G.J. Hallman (eds.) pages 225-237, Westview Press, Boulder CO.

6. Liquido, N.J., R.L. Griffin, and K.W. Vick. 1997. Quarantine Security for Commodities: Current Approaches and Potential Strategies. Proc. Of Joint Worskshops of the Agricultural research Service and the Animal and Plant Health Inspection Service. June 5-9 and July 31-Aug 4, 1995. Beltsville, MD.

7. Mangan, R.L. and J.L. Sharp. 1994. Combination of multiple treatments. In Quarantine Treatments for Pests of Food Plants, Sharp, J.L. and G.J. Hallman (eds.) pages 239-247, Westview Press, Boulder CO.

8. Miller, C.E., A.S. Green, V. Harabin, and R.D. Stewart. 1995. A Systems approach for Mexican Avocado – Risk Management Analysis. USDA APHIS. 27pp.

9. Robertson, J.L. and H.K. Preisler. 1992. Pesticide bioassays with arthropods. Boca Raton, Florida:CRC Press.